

CONFIRMATION?

Gel electrophoresis

inderny gel = good

indirect vector: 6123456

$\overline{MC1P}$ $\overline{MC2P}$ $\overline{MC3P}$ $\overline{MC4P}$ $\overline{MC5P}$ $\overline{MC6P}$ $\overline{MC1N}$ $\overline{MC2N}$ $\overline{MC3N}$

res. neg. MC4N MC5N MC6N MC7N MC8N MC9N MC10N MC11N MC12N MC13N MC14N MC15N MC16N MC17N MC18N MC19N MC20N MC21N MC22N MC23N MC24N MC25N MC26N MC27N MC28N MC29N MC30N MC31N MC32N MC33N MC34N MC35N MC36N MC37N MC38N MC39N MC40N MC41N MC42N MC43N MC44N MC45N MC46N MC47N MC48N MC49N MC50N MC51N MC52N MC53N MC54N MC55N MC56N MC57N MC58N MC59N MC60N MC61N MC62N MC63N MC64N MC65N MC66N MC67N MC68N MC69N MC70N MC71N MC72N MC73N MC74N MC75N MC76N MC77N MC78N MC79N MC80N MC81N MC82N MC83N MC84N MC85N MC86N MC87N MC88N MC89N MC90N MC91N MC92N MC93N MC94N MC95N MC96N MC97N MC98N MC99N MC100N MC101N MC102N MC103N MC104N MC105N MC106N MC107N MC108N MC109N MC110N MC111N MC112N MC113N MC114N MC115N MC116N MC117N MC118N MC119N MC120N MC121N MC122N MC123N MC124N MC125N MC126N MC127N MC128N MC129N MC130N MC131N MC132N MC133N MC134N MC135N MC136N MC137N MC138N MC139N MC140N MC141N MC142N MC143N MC144N MC145N MC146N MC147N MC148N MC149N MC150N MC151N MC152N MC153N MC154N MC155N MC156N MC157N MC158N MC159N MC160N MC161N MC162N MC163N MC164N MC165N MC166N MC167N MC168N MC169N MC170N MC171N MC172N MC173N MC174N MC175N MC176N MC177N MC178N MC179N MC180N MC181N MC182N MC183N MC184N MC185N MC186N MC187N MC188N MC189N MC190N MC191N MC192N MC193N MC194N MC195N MC196N MC197N MC198N MC199N MC200N MC201N MC202N MC203N MC204N MC205N MC206N MC207N MC208N MC209N MC210N MC211N MC212N MC213N MC214N MC215N MC216N MC217N MC218N MC219N MC220N MC221N MC222N MC223N MC224N MC225N MC226N MC227N MC228N MC229N MC230N MC231N MC232N MC233N MC234N MC235N MC236N MC237N MC238N MC239N MC240N MC241N MC242N MC243N MC244N MC245N MC246N MC247N MC248N MC249N MC250N MC251N MC252N MC253N MC254N MC255N MC256N MC257N MC258N MC259N MC260N MC261N MC262N MC263N MC264N MC265N MC266N MC267N MC268N MC269N MC270N MC271N MC272N MC273N MC274N MC275N MC276N MC277N MC278N MC279N MC280N MC281N MC282N MC283N MC284N MC285N MC286N MC287N MC288N MC289N MC290N MC291N MC292N MC293N MC294N MC295N MC296N MC297N MC298N MC299N MC300N MC301N MC302N MC303N MC304N MC305N MC306N MC307N MC308N MC309N MC310N MC311N MC312N MC313N MC314N MC315N MC316N MC317N MC318N MC319N MC320N MC321N MC322N MC323N MC324N MC325N MC326N MC327N MC328N MC329N MC330N MC331N MC332N MC333N MC334N MC335N MC336N MC337N MC338N MC339N MC340N MC341N MC342N MC343N MC344N MC345N MC346N MC347N MC348N MC349N MC350N MC351N MC352N MC353N MC354N MC355N MC356N MC357N MC358N MC359N MC360N MC361N MC362N MC363N MC364N MC365N MC366N MC367N MC368N MC369N MC370N MC371N MC372N MC373N MC374N MC375N MC376N MC377N MC378N MC379N MC380N MC381N MC382N MC383N MC384N <

on 10/10/16 140616 RBC & albumin

Negatives and Positives

EXPECTED RESULT:

* Second PCR of Primers & 363 with new mCherry templates attempting to amplify @ 65°C

(☒ check if APE file exists on Drive)

- * Did RBS primer PCR at 65°C
- ↳ Used Template 1 (conc. ~~2023~~ 2023) (mCherry)

- ★ Min: prep military cultures.

↓ heat inactivate enzymes.

POSITIVE: psc - 1e3 - in Cherry → 2k6 band & 700bp band. ✓

6/16: PCR

Digest: RBS primers (VF) 074-079

Settings: 65°C Program

Negative: No polymerase.

Name: Kathryn Fittan

Date: 6/16/14

CONFIRMATION?

thing gel

WEEKLY ONE SENTENCE:

continue testing RBS primers/promoter

GEL LANE ORDER:

DAILY ONE SENTENCE:

~~owl~~ which enzymes (Hsd) → ok: initiation day

digest plasmid's RBS primer/promoters (prim)

ligate together, mini-prep mcherry cultured

METHOD(S) OF CHOICE:

digestion w/ X & P

ligation gel extraction TRANSFORM!

EXPECTED RESULT:

✓ plasmid digest → ~~2 kb~~ 2 kb; 700 bp

✓ insert digest → ~~700~~ 700-800 bp

ligation → 2.8 kb ✓

(☒ check if APE file exists on Drive)

TEMPLATE(S): mcherry ✓

OLIGO(S): —

THERMOCYCLER SETTINGS: 1 hr @ 37°C; 20 min @ 80°C; -A forever ✓

CONTROLS:

POSITIVE:

~~2 kb~~ digested mcherry
700 & 2 kb band ✓

NEGATIVE:

w/o enzymes ✓

GEL PICTURE / OTHER SPACE:

Name: Jennifer Zhang

Date: 6/10/14

WEEKLY ONE SENTENCER: continue testing the RBS primer / promoter pairs

DAILY ONE SENTENCER: digest plasmid and our RBS primer / promoter insert and ligate

which?

METHOD(S) OF CHOICE:

plasmid / insert digestion (w/ xhoI and ~~pstI~~)

gel extraction

ligation

transformation

EXPECTED RESULT:

plasmid digest → ~1.5 kb

insert digest → ~1.5 kb

ligation → ~2.5 kb

(X) check if APE file exists on Drive)

TEMPLATE(S): [mcherry RBS primer / promoter w/] which template

OLIGO(S): —

THERMOCYCLER SETTINGS: 1hr - 37°C ; 20min - 80°C ; 4°C - forever

CONTROLS:

POSITIVE:

psbC3-mcherry → 700 bp ; 2 kb band

NEGATIVE:

φEnzymes

CONFIRMATION?

GEL LANE ORDER:

mcherry Vector: L 1 2 3 4 5 6

GEL PICTURE / OTHER SPACE:

• miniprep mcherry cultures OK!

• 6/10 PCR

template: miniprep mcherry 1 (207.5 ng/mL)

oligos: RBS primers (VF) 014 - 079

settings: 0303 RBS05

program

negative: no polymerase

→ gel extracted all lanes except RL2

→ reran RBS promoter PCR w/ the gel

→ extracted products from previous RBS promoter PCR

as templates

→ 65°C

Name: Coleen Tran

Date: 6/16/14

WEEKLY ONE SENTENCER: redo RBS/promoter combinations

DAILY ONE SENTENCER: ~~redo~~ digest + ligate
RBS/promoters into mCherry vector

which?
P_{Hz} RBS

METHOD(S) OF CHOICE:

digest w/ X and P

EXPECTED RESULT:

plasmid \Rightarrow 2.8 kb \checkmark undigested; 2 kb digested
700 bp digested \rightarrow mCherry

(☒ check if APE file exists on Drive) \checkmark

TEMPLATE(S): mCherry + RBS/promoter which??

OLIGO(S):

THERMOCYCLER SETTINGS: 1st = 37°C; 20' - 80°C ; 4°C

CONTROLS:

POSITIVE:

mCherry template w/ X+P
(mCherry!)

NEGATIVE:

no enzyme

CONFIRMATION?

gel

GEL LANE ORDER:

L MCP MCP MCP MCP MCP
1 2 3 4 5 6

RBS + mCherry

MCP MCP MCP MCP MCP

GEL PICTURE / OTHER SPACE:

On google drive

140616 RBS + mCherry Gel Purify

Today: PCR w/ new mCherry template (gel confirmation on Google Drive) + RBS primer

